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Contractor: BIONETICS RESEARCH LABORATORIES, Inc.

Contract No.: DA 18-108-AMC-119(A)

THIRD QUARTERLY PROGRESS REPORT

Covering the Period

23 September 1963 - 22 December 1963

Title: New Methods Development for Irritant Screening

Prepared by

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INTRODUCTION

This third quarterly progress report presents a statement of work accomplished during the period from 23 September 1963 to 22 December 1963 by the Contractor, Bionetics Research Laboratories, Inc., under Contract No. DA 18-108-AMC-119(A).

Before proceeding to a statement of accomplishment during the report period, it seems appropriate to call attention to the fact that during this period substantial modifications in the scope of the original contract have occurred. A modification of the contract became effective on 12 November 1963 calling for an increase in the effort devoted to assessment of irritant potential by existing methods, so that a total of approximately 280 compounds could be evaluated within the third quarter. Since this modification did not alter the total funds allocated to the program, the additional work could be accommodated only by a marked reduction in the level of effort on the methods development phase of the project. This has, of course, had a marked influence on the accomplishments which are presented below. A second modification, effective 23 December 1963, restores the balance between the two phases of the Contract and increases the term and total effort on each phase. Subsequent reports can, therefore, be expected to relate much more substantial progress.

The balance of this report is divided into sections which correspond to the sections of the Statement of Work of the Contract. Thus, Section I (New Methods Development) discusses the progress which has been made in that phase of the work. Section II (Assessment of Irritant Potential) discusses the screening operations under the corresponding phase of the work.

SECTION I - New Methods Development

The changed objectives of the project have reduced the time devoted to this phase to somewhat less than half of that originally planned.

A. Work Accomplished.

Histamine liberation

As previously explained, the first proposed new method to be explored in this Contract is a possible correlation between histamine liberation and irritant potential. For this purpose "tissue" is incubated with various irritants and the amount of histamine liberated is determined fluorimetrically. Upon the suggestion of our consultant, Dr. V. H. Cohn, the first "tissue" investigated has been mast cells from the peritoneum of rats (for methodology see Second Quarterly Report). The difficulties previously reported have been confirmed by further survey of conditions of incubation. We now conclude that the total amount of histamine liberated from rat peritoneal mast cells is insufficient and the differences between irritants too slight to justify further effort in this direction. The various devices reported by other workers for circumventing these difficulties such as microscopic observation of cell rupture and biological assay for histamine using isolated guinea pig ileum, are not appropriate for our particular application.

Before complete abandonment of efforts to correlate irritation with histamine liberation, it was decided to investigate a tissue which provides a substantially larger amount of available

histamine. The lungs of guinea pigs have been investigated and appear to be suitable. A washed mince of lung tissue in Tyrode's solution is incubated 60 minutes with the test compound in concentrations varying from experiment to experiment over the range of 0.01 to 1.0% (W/V). A supply of Compound 48-80 was obtained for use as a positive control through the courtesy of Dr. John V. Burns, Director of Research of Burroughs Wellcome & Company.

Histamine release has been observed with 48-80 and with hexamethylene diisocyanate, but not with CS. These results are encouraging, but not yet conclusive. The limitation of time and funds during the report period has precluded performance of the experiments necessary to evaluate properly the relative activity of the test compounds, especially in the light of problems resulting from their low water solubility and the consequent necessity for inclusion of nonaqueous solvents. Thus, a series of control experiments with solvents such as glycols is needed. In addition, 48-80 is so powerful in liberating histamine that less active positive control compounds are desirable. We are investigating N-octylamine and N-decylamine for this purpose.

Species survey

It was previously reported that mice are unsuitable for the study of eye irritants because of their small size and the consequent difficulty in observation.

The reported tentative conclusions with respect to the suitability of dogs remain valid after additional investigation. Three compounds - EA 3305, hexamethylene diisocyanate and CS 43038 were chosen as representative of mildly, moderately and severely irritating compounds in rabbits and were applied to dogs in the same concentrations used in rabbits. We conclude, more firmly than before, that: (1) the dog is somewhat less sensitive to eye irritation than is the rabbit; (2) the usual dark-colored iris in the dog makes examination somewhat more difficult and (3) the combination of blepharospasm and extension of the nictitating membrane which occurs in some dogs in the presence of irritation makes it difficult or occasionally even impossible to observe the iris and cornea. To these may be added another consideration that: (4) the increased size and greater cost of dogs represent disadvantages which though not substantial are not counterbalanced by advantages. Thus, it appears that the dog should be rejected as a substitute for rabbits in the study of eye irritant potential.

A start has been made on the study of the sensitivity of the rhesus monkey eye to irritants. In the two animals thus far tested with a single compound (CS 31533), the spectrum of signs observed has been closely comparable to those found in rabbits. However, the intensity of the effects seem in general to be somewhat less and recovery occurs somewhat more rapidly. Additional animals and additional compounds must be studied before any conclusions are justified.

B. Plans for subsequent period.

The necessary additional experiments for the evaluation of histamine liberation as indicated above will be performed.

The species survey will continue. Additional work will be performed on monkeys including species other than the rhesus. Cats will also be studied.

The plans mentioned in the previous report to initiate investigation of ciliary activity and tracheal smooth muscle have been held in abeyance due to the modification of the Contract. These will now be reinstituted and results can be expected within the next report period.

Considerable thought is being devoted to the development of a behavioral test for irritation. For this type of testing it will be necessary to develop some acceptable means of generating and delivering an aerosol of the irritant. While several means of accomplishing this have been suggested, none of these have been pursued sufficiently to warrant comment at this point.

SECTION II - Assessment of Irritant Potential

A. Compounds received:

Received October 8, 1963

CS 29780	CS 42068	CS 42692	CS 43029	CS 6133
CS 29936	CS 42296	CS 42693	CS 43038	CS 9294
CS 29944	CS 42302	CS 42694	CS 43058	CS 20220
CS 30006	CS 42306	CS 42697	CS 43062	CS 20221
CS 30373	CS 42307	CS 42702	CS 43085	CS 20222
CS 30441	CS 42310	CS 42703	CS 43111	CS 20246
CS 30482	CS 42311	CS 42706	CS 43112	CS 20247
CS 30518	CS 42312	CS 42718	CS 43117	CS 20259
CS 31209	CS 42315	CS 42720	CS 43128	CS 20267
CS 31304	CS 42319	CS 42721	CS 43146	CS 20269
CS 31325	CS 42345	CS 42722	CS 43147	CS 22656
CS 31326	CS 42641	CS 42728	CS 43154	CS 22657
CS 31513	CS 42642	CS 42729	CS 43301	CS 22658
CS 31531	CS 42646	CS 42736	CS 43322	CS 22686
CS 31533	CS 42647	CS 42738	CS 43323	CS 22698
CS 31701	CS 42648	CS 43004	CS 43325	CS 22699
CS 31738	CS 42649	CS 43006	CS 43327	CS 22704
CS 31740	CS 42650	CS 43007	CS 43328	CS 22712
CS 31755	CS 42654	CS 43009	CS 43329	CS 22744
CS 31760	CS 42656	CS 43010	CS 43330	CS 23653
CS 31763	CS 42658	CS 43011	CS 43331	CS 24170
CS 36811	CS 42663	CS 43013	CS 43375	CS 27474
CS 39469	CS 42665	CS 43014	CS 43382	CS 28844
CS 39470	CS 42669	CS 43019	CS 43167	CS 28955
CS 39711	CS 42675	CS 43023		CS 29338
CS 39715	CS 42679	CS 43025		CS 29764
CS 42065	CS 42688	CS 43026		CS 29779

Received December 20, 1963

CS 6556	CS 33231	CS 33659	CS 44522	CS 44860
CS 11470	CS 33260	CS 39453	CS 44523	CS 44861
CS 18170	CS 33272	CS 39515	CS 44530	CS 44863
CS 18108	CS 33326	CS 39523	CS 44531	CS 44864
CS 18109	CS 33335	CS 39524	CS 44548	CS 44881
CS 18110	CS 33347	CS 39525	CS 44851	CS 44882
CS 18175	CS 33359	CS 42546	CS 44853	CS 44883
CS 18454	CS 33360	CS 43598	CS 44854	CS 44884
CS 32716	CS 33403	CS 43603	CS 44855	CS 44885
CS 32718	CS 33410	CS 43604	CS 44856	CS 44886
CS 32811	CS 33434	CS 43608	CS 44857	CS 44887
CS 33225	CS 33657	CS 44521	CS 44859	CS 44888
CS 44889	CS 45013	CS 45246	CS 45420	CS 45646
CS 44891	CS 45014	CS 45251	CS 45429	CS 45651
CS 44938	CS 45015	CS 45253	CS 45442	CS 45652
CS 44943	CS 45016	CS 45256	CS 45443	CS 45659
CS 45003	CS 45017	CS 45401	CS 45506	CS 45660
CS 45004	CS 45018	CS 45402	CS 45508	CS 45663
CS 45005	CS 45019	CS 45403	CS 45509	CS 45664
CS 45006	CS 45020	CS 45404	CS 45510	CS 45665
CS 45008	CS 45021	CS 45405	CS 45511	CS 45947
CS 45010	CS 45223	CS 45408	CS 45512	CS 33689
CS 45011	CS 45234	CS 45410	CS 45513	CS 45247
CS 45012	CS 45245	CS 45414	CS 45514	

B. Work completed:

Assessment has now been completed on the following 33 compounds which were incomplete at the time of the second quarterly report:

CS 39654	CS 43054	CS 41592
CS 40685	CS 28938	CS 42214
CS 40797	CS 39242	CS 42218
CS 40843	CS 40834	CS 42811
CS 41723	CS 40841	CS 42821
CS 42039	CS 40849	CS 42824
CS 42355	CS 40850	CS 42998
CS 42984	CS 41460	CS 43000
CS 42987	CS 41462	CS 43169
CS 42990	CS 41543	CS 42737
CS 42997	CS 41576	CS 41593

Complete assessment has been accomplished on the following 121 compounds:

CS 20220	CS 31755	CS 43038	CS 23412	CS 40775
CS 20222	CS 31760	CS 43062	CS 25257	CS 40777
CS 22657	CS 39470	CS 43085	CS 25657	CS 40787
CS 22686	CS 39711	CS 43112	CS 27644	CS 40789
CS 22698	CS 42065	CS 43128	CS 29231	CS 40795
CS 22699	CS 42068	CS 43146	CS 31628	CS 40796
CS 22704	CS 42345	CS 43147	CS 31639	CS 40877
CS 22744	CS 42641	CS 43154	CS 31676	CS 41458
CS 23653	CS 42654	CS 43301	CS 31688	CS 41724
CS 24170	CS 42658	CS 43325	CS 36845	CS 41727
CS 27474	CS 42665	CS 43327	CS 38358	CS 42054
CS 28844	CS 42669	CS 43329	CS 38359	CS 42064
CS 28955	CS 42720	CS 43330	CS 38363	CS 42066
CS 29338	CS 42722	CS 43331	CS 38365	CS 42069
CS 29764	CS 42729	CS 43375	CS 38366	CS 42224
CS 29780	CS 43004	CS 43382	CS 39062	CS 42354
CS 29944	CS 43010	CS 3648	CS 39229	CS 42356
CS 30006	CS 43011	hexamethylene	CS 39230	CS 42982
CS 30373	CS 43013	diisocyanate	CS 39232	CS 42983
CS 30441	CS 43014	EA 3305	CS 39651	CS 43157
CS 31325	CS 43019	CS 7846	CS 39666	CS 43175
CS 31533	CS 43023	CS 21071	CS 39887	CS 43178
CS 31701	CS 43025	CS 21640	CS 40322	CS 43179
CS 31738	CS 43026	CS 21682	CS 40667	CS 43180
			CS 40670	92649

The following 31 Compounds have been evaluated for skin irritation only:

CS 20246	CS 42307	CS 42702
CS 20247	CS 42311	CS 42718
CS 29936	CS 42319	CS 42736
CS 30518	CS 42646	CS 42812
CS 31626	CS 42647	CS 43006
CS 31740	CS 42663	CS 43009
CS 31763	CS 42675	CS 43322
CS 39469	CS 42679	CS 43323
CS 40678	CS 42688	CS 43328
CS 42302	CS 42692	
CS 42306	CS 42693	

One Compound has been evaluated for eye irritation only:

CS 36811

Attention is called to an error which has been discovered in the second quarterly report. Partial assessment (evaluated for eye irritation only) was reported for Compound CS 42747. Compound should have been CS 42737. Assessment for this compound is now complete, and has been so reported.

C. Future plans.

The second modification of the Contract provides for evaluation of a cumulative total of 600 compounds. No difficulty is foreseen in accomplishing this goal well within the Contract period.

SECTION III - Summary

During the period rat peritoneal mast cells have been found to liberate too small a quantity of histamine to permit evaluation of the activity of irritants. Guinea pig lungs appear suitable, but the results are still inconclusive.

Mice and dogs appear inferior to rabbits for evaluation of eye irritation. Work with monkeys is in progress, but no conclusions are yet justifiable.

Assessment by current methods has now been accomplished on a cumulative total of approximately 280 compounds.

AD	Accession No.	UNCLASSIFIED	AD	Accession No.	UNCLASSIFIED
Bionetics Research Laboratories, Inc.			Bionetics Research Laboratories, Inc.		
Falls Church, Va.			Falls Church, Va.		
NEW METHODS DEVELOPMENT FOR IRRITANT			NEW METHODS DEVELOPMENT FOR IRRITANT		
SCREENING - E. Ross Hart			SCREENING - E. Ross Hart		
Report No. 3, 22 December 63, 10 pp			Report No. 3, 22 December 63, 10 pp		
Contract DA 18-108-AMC-119(A)			Contract DA 18-108-AMC-119(A)		
4C08-03-016-07			4C08-03-016-07		
Rat peritoneal mast cells liberate			Rat peritoneal mast cells liberate		
insufficient histamine/or evaluation			insufficient histamine/or evaluation		
of irritants; guinea pig lungs appear			of irritants; guinea pig lungs appear		
suitable. Mice and dogs inferior to			suitable. Mice and dogs inferior to		
rabbits for study of eye irritation;			rabbits for study of eye irritation;		
monkeys are being studied. Assessment			monkeys are being studied. Assessment		
by current methods now complete on			by current methods now complete on		
approximately 280 compounds.			approximately 280 compounds.		
UNCLASSIFIED			UNCLASSIFIED		
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by current methods now complete on			by current methods now complete on		
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monkeys are being studied. Assessment			monkeys are being studied. Assessment		
by current methods now complete on			by current methods now complete on		
approximately 280 compounds.			approximately 280 compounds.		
UNCLASSIFIED			UNCLASSIFIED		